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Review

Immunopathology and oligoclonal T cell expansions. Observations in immunodeficiency, infections, allergy and autoimmune diseases

Vitor Pordeus[1,*], Gustavo C. Ramos[2], Claudia R. Carvalho[3], Archimedes Barbosa De Castro Jr.[4], Andre Pires Da Cunha[4] and Nelson M. Vaz[4]

[1]Núcleo de Cultura, Ciência e Saúde da Secretaria Municipal de Saúde e Defesa Civil, Rio de Janeiro, [2]Departamento de Farmacologia, CCB, UFSC, Florianópolis, SC, [3]Departamento de Morfologia, ICB, UFMG, Belo Horizonte, MG, [4]Departamento de Bioquímica e Imunologia, ICB, UFMG, Belo Horizonte, MG, Brasil

ABSTRACT

The immune system is usually seen as a collection of independent (specific) lymphocyte clones. Randomly generated and activated at random, these lymphocytes follow only their individual, clonal history. Thus, in traditional descriptions, immunological activity is neither systemic nor historical and is never "physiological". However, recent descriptions show an abundant "auto"-reactivity in healthy organisms, an evidence of internal connectivity. The two major sources of immunogenic contacts, namely, dietary proteins and products of the autochthonous microbiota fail to induce progressive "secondary-type" clonal expansions (or "memory"). Natural IgM may arise in "antigen-free" organisms as they do in conventionally raised animals; actually, clonal receptors of both T and B lymphocytes are formed in antigen-free intracellular environments and are not driven by antigen exposure. Early in ontogenesis natural immunoglobulins are organized in characteristic patterns of reactivity which are robustly stable throughout healthy living; these patterns depend on genes known to be important in immunological activity. Predictable (not-random) variations on these patterns occur during infectious and autoimmune diseases, both in humans and experimental animals, which are correlated with different clinical states of these diseases. All this is incompatible with a random process driven by independent lymphocytes. In different pathological conditions, ranging from immunodeficiencies to parasite, allergic and autoimmune diseases, the organism develops oligoclonal expansions of T lymphocytes. In addition, oligoclonality is associated with high IgE titers and eosinophilia. We propose, therefore, that the physiology of the immune system is conservative and remains stable throughout healthy living. In several types of experimental and clinical diseases, this stability is broken by oligoclonal expansions of T cells. Specific immune responses, understood as the progressive expansion of oligoclonal lymphocytes, are expressions of immunopathology rather than immune physiology. A new explanation of the protective effects of anti-infectious vaccination is offered.

KEYWORDS: theory, immune system, conservation, oligoclonality, vaccines

***Corresponding author**
Dr. Vitor Pordeus
Rua Prof. Antônio Maria Teixeira, 33 apto 203,
Leblon, Rio de Janeiro, Brazil 22430050
vitorpordeus@gmail.com

INTRODUCTION

A glance through history

It is trite to say that one may not explain pathologic deviations if we ignore the physiology of which they are deviations. Current immunology, neglects the need to define a physiology for the immune system, its behavior in healthy living [1-3]. Immunology is historically linked to the study of diseases: first infections, then allergies and, from the 1960s, pathogenic autoimmunity. New experimental developments, however, may allow us to understand what the immune system does in healthy living and, based on this knowledge, trace common mechanisms of infectious, allergic and autoimmune diseases.

By the end of 19th century, Pasteur and Koch developed a theoretical framework, known as the 'Germ Theory' of infectious diseases [4] and immunologists devoted themselves to study vaccines and other immune defenses against infectious agents. Vaccination and serum therapy were invented as successful forms of prevention and treatment of infectious diseases. The first molecular and cellular components of immunological activity- namely, specific antibodies, the Complement-system, macrophages and phagocytosis- were characterized in the pursuit to understand how an organism becomes specifically "immune" to a particular pathogen and how the "memory" of this event may be conserved in the organism.

Meanwhile, "natural" or spontaneously formed antibodies of unknown origin, such as human isohemagglutinins, were also characterized [5] and severe hypersensitivity reactions, such as anaphylaxis [6] and serum sickness [7] were shown to be triggered by immunological mechanisms. These findings blurred the exclusive "anti-infectious" role played by specific immune mechanisms. The formation of specific antibodies to plant proteins as well as to allelic variations of red cells of the same species was demonstrated [8] and diseases that are presently recognized as autoimmune, such as Paroxysmal Nocturnal Hemoglobinuria, were also characterized at that moment [9].

In the following period, the enthusiasm was curtailed because inventing effective new vaccines and antisera proved to be much harder than anticipated. Research in Immunology was diverted to biochemical (immunochemical) trends. On the one hand, this allowed fundamental progress to be made, such as unraveling the basic physicochemical structure of specific antibodies, their antigen binding sites, variable and constant regions, etc. On the other hand, the interest was kept distant from biological problems [4]. The ("adoptive") transfer of cell-dependent immunologic phenomena with living cells was only recognized in the mid 1940s [10]; the thymus and T lymphocytes were characterized in the 1960s [11, 12]; and the role of the MHC in T cell activation only in the 1980s [13, 14].

In summary, in the first half of the last century, Immunology developed under a biochemical and medical orientation. During this period, the notion of "allergy" as a deviant, pathogenic form of inflammation was developed, and, especially in Germany, chronic diseases were understood as distortions in regenerative processes. This was quite different and did not include or correspond to what, from the 1960's on, was understood as forms of lymphocyte-dependent "autoimmune" damage [15]. "Cellular Immunology" [16], was born in the 1950-1960s. Lymphocytes were definitely brought to a central position [17, 18].

Two unexpected turns then took place: specific immunological "tolerance" was described in studies of allogenic skin grafts [19]; and Jerne proposed the Natural Selection Theory of Antibody Formation [20]. Soon thereafter, Burnet proposed The Clonal Selection Theory (CST) as a cellular modification of Jerne's proposal [21, 22].

The CST claimed that in response to their specific antigens, immunological activity arises by the expansion of lymphocytes into "clones" - lymphocyte collections with the same genetic (nucleic) content, which then expand and differentiate into antibody-secreting cells. The theory explained specific immune responses, immunological memory and, thus, their putative role in anti-infectious vaccination. In addition, the CST explained the phenomenon of specific immunological "tolerance" to alloantigens, suggesting that it was due to specific clonal abrogation. As the CST proposed a random origin for lymphocyte clones, "auto-reactive" clones

would necessarily arise and should be "forbidden" to expand in order to avoid "auto-aggressions" (autoimmune diseases). Thus, self/nonself discrimination and pathogenic autoimmunity became central tenets in the theory. Experimental models of such autoimmune aggressions were soon developed [23] and immediately thereafter clinical counterparts were also described [24]. The concept of auto-immunity underwent a huge development in medicine; presently, more than 80 different diseases are described as "autoimmune" and the idea of pathogenic autoimmunity replaced the idea of "allergy" [15] as the main mechanism involved in chronic human diseases.

The idiotypic network: A frustrated systemic approach

A second wave of developments not directly related to infections and anti-infectious defense as the *raison d'être* of immunological activity, occurred in the 1970s. Although widely accepted, the CST has a major flaw: it forbids auto-reactivity and, in so doing, excludes the possibility of interclonal connections and also interactions of these clones with the organism. In other words, forbidden auto-reactive clones preclude all kinds of systemic – organism-centered – approaches to immunological activity.

The Idiotypic Network Theory offered a brand new understanding to immunological activity in which auto-reactivity was turned from forbidden activity into physiological rule [25]. Although frequently neglected by mainstream immunologists, especially in United States [26], idiotypes have a clear relevance in the development of the immune system [27], of specific memory [28] and of immunopathology [29]. The network approach allowed the description of an internal, immanent source of immunological activity, as a first approach to a physiology of the system [1, 30]. Lymphocytes are certainly involved in physiological activities, such as dietary protein assimilation [31, 32]; pregnancy and lactation [27]; tissue regeneration [33]; cell turnover and apoptosis [34] - among many other phenomena.

The conservative physiology of the immune system

Stemming from immunological phenomena triggered by mucosal exposures to antigens, we recently proposed that the immune system exhibits a conservative physiology [1, 3]. It is widely accepted that the feeding of a given antigen is capable of "decreasing" rather than increasing B and T cell responsiveness to it, a phenomenon called "oral tolerance" [35, 36]. However, rather than a decrease in immunological responsiveness, oral tolerance is more correctly described as a stabilization of lymphocyte reactivity, that 'locks' robust patterns of antibodies formation in spite repeated parenteral immunizations with a given immunogen in adjuvant [37]. The physiological activity of mucosal lymphocytes is a most prominent aspect of immunological activity, since the number of lymphocytes in the small intestine exceeds several fold the number found in all the other lymphoid organs together [38]; in addition, hundreds of grams of dietary proteins are placed in contact with the human small intestine every day, during our healthy, physiological daily feeding, and some of it penetrates the body in immunologically relevant forms [35, 36]. In addition, the analysis of the reactivity of 'natural antibodies' (natural serum IgM and IgG), through a modified technique of immunobloting [39, 40] and also of T cells, through spectratyping (CDR3 length analysis) [41], repeatedly showed that patterns of reactivity of B and T lymphocytes with complex antigen mixtures are robustly conserved throughout the healthy living of human beings and a variety of experimental animals [42]. These patterns are established early in ontogenesis- around 2 years-old humans- and remain conserved throughout healthy living. Similar findings regarding the stability of T cell repertoires have been described with spectratyping [43] and V-beta repertoire analysis by flow cytometry [44]. In elder humans, more consistently at the seventh decade, the pattern of reactivity of immunoglobulins and T cells begin to change and this coincides with the handicaps associated with immunossenescence [45].

In short, when tested *en bloc*, B and T lymphocytes of healthy organisms display remarkably stable patterns of reactivity, reflecting the activity of a robust network of lymphocytes. Mucosal contacts with immunogens, a daily event of highly physiological significance, also leads to stable levels of specific reactivity, rather than a

progressive (memory-type) reactivity [37]. These events derive from the conservative physiology of the immune system [1] which, when seriously considered, may open the possibility to explain immunopathological deviations in infectious, allergic and autoimmune diseases, as well as the relations between them.

Patterned deviations in infectious, allergic and autoimmune diseases

Traditionally, immunologists have been deeply motivated with quantitative aspects of specific antibodies production and T cell activation, whereas the diversity of lymphocytes involved in each case has been neglected. It would seem that a pronounced expansion of few lymphocytes clones would be equivalent to the moderate expansion of many clones. However, whereas the physiological patterns of immunoglobulins and TCR repertoires aforementioned that have been characterized in healthy individuals derived from the activity of all the lymphocytes in the body [42-44], oligoclonal expansions of T cells have also been characterized in a wide range of infectious [45] and autoimmune diseases [46].

Omenn's syndrome, a severe congenital human anomaly, generally fatal in the first few months of life, is an outstanding example of a abnormal development of T lymphocytes, which also involves Langerhans cells, eosinophils and an intense synthesis of IgE. In this condition, generally, the thymus and lymph nodes are emptied of lymphocytes [47, 48] but the mutations affecting Rag-1 or Rag-2 don't block lymphopoiesis totally and a few clones of T lymphocytes are activated and expand to form an oligoclonal repertoire [49, 50]. Somehow, this oligoclonality is important in the pathogenesis of Omenn's syndrome, which includes high eosinophilia and an intense production of IgE.

A recent experimental example makes the association between increased production of IgE and T cell oligoclonality extremely evident. Rag-knockout (Rag-KO) mice were produced to contain exclusively monoclonal populations of B and T lymphocytes reactive respectively with hemagglutinin of the influenza virus (HA) and peptides from hen's ovalbumin (OVA). A single immunization of these "bi-clonal" mice with an OVA-HA conjugate resulted in a production of IgE hundred-fold higher than normal (30-200 μg/ml). This increased IgE production could be avoided by the infusion of normal syngeneic T CD4+ policlonal lymphocytes, either CD25+ or CD25-. These data suggest that in normal individuals, IgE production is controlled by the policlonal activity of CD4+ T cells [51].

Omenn's syndrome may also be seen as a natural counterpart of a series of experiments investigating the consequences of thymectomy of 3-day old mice [52, 53]. Thymectomy at this early stage is pathogenic because it allows the introduction of a sub-optimal variety of T in lymphopenic organisms, and these cells undergo extensive expansion. The variety of T cells emerging from the thymus in the first three days extra-uterine of mouse life is insufficient to establish normal T cell diversity; when these oligoclonal lymphocytes expand, they become pathogenic [52, 53]. This expansion is independent of the recognition of external antigens, but depends on the recognition of MHC-linked autologous peptides [54]; it may be as high as thirty-fold, is biased in the type of beta (V-beta) chain used in the TCR, as shown by immunoscope analysis which show a decrease in the heterogeneity of the distribution of the length of the (CDR)3 region [55].

Omenn's syndrome as well as the phenomena described by Sakaguchi [53] and de Lafaille [51] are examples of pathogeny derived from an incompleteness of the immune system. This phenomenon may also be expressed in numerous examples in clinical literature, which we also shall briefly discuss.

The pathogenesis of atherosclerosis, the leading cause of death worldwide, a process underlying myocardial infarction and stroke, is also associated to a skewed repertoire of immunoglobulins [56] and the presence of oligoclonal intralesional T cells in mice [57] as well as in human beings [58]. A higher degree of oligoclonality was seen in Acute Coronary Syndrome, suggesting that it may be a maker of plaque instability [59].

T cell oligoclonal expansions, particularly of CD8 T lymphocytes, have been associated to normal aging. Elder people have important immunological abnormalities, named Immune Risk Phenotype [60],

with increased frequency of infections, autoimmune, degenerative diseases and a higher mortality in two years follow-up. These oligoclonal expansions have been linked to chronic viral infections by cytomegalovirus (CMV) [61] and Epstein-Barr virus (EBV) [62] which occurs during young age and persist throughout life [63]. It has been proposed that EBV-infected B cells become long-living and become responsible for the chronic stimulation of T cells and their oligoclonal expansion, which along with individual genetic predisposition (HLA haplotypes) would explain the association of different autoimmune diseases to EBV infection [64]. In another model, neonatal infection with attenuated lymphocytic chorio-meningitis virus (LMCV), which also leads to long-lasting infection, leads to severe encephalitis upon re-infection with the wild type virus, a phenomenon called "viral *déjà vu*" [65]. Finally, in chronically infected HIV patients, a higher degree of oligoclonality of T CD4 cells is correlated to low CD4 counts on peripheral blood, a known factor of immunodeficiency progression in this clinical setting [66].

Several observations have shown that an infection may stimulate the same T cell clones involved in an "autoimmune" phase of disease. For instance, rheumatic heart disease exhibits the same oligoclonal TCR that interact with streptococcal M protein and are found in heart infiltrating lymphocytes [67]. Similarly, it has been reported that tonsil infiltrates from streptococcal angina patients exhibit the same T cell clones of skin infiltrates from psoriasis vulgaris patients [68]. During common infections, oligoclonality of T cells do occur [69], but, for the vast majority of individuals, robust mechanisms drive the immune system back to the physiological stable state. Otherwise, when sustained or progressive oligoclonal expansions occur, they are involved in autoimmune diseases, or, alternatively, if a microorganism is identified within the process, it is usually pointed out as the culprit of a chronic infection.

Sustained T cell oligoclonality

The remarkable degeneracy of the T cell receptors [70] may be involved in oligoclonal T cell responses in infections with accompanying autoimmune pathology. Degeneracy means that a single T cell receptor is able to interact with as many as 10^9 different ligands as demonstrated *in vitro* [71]. As mentioned above, molecular mimicry may link some autoimmune diseases and infectious diseases, such as Rheumatic Fever and *Streptococcus pneumoniae* beta hemolytic group A infection [72], or Chagas' disease and *Trypanosoma cruzi* infection [73]. In Antiphospholipid Syndrome (APS), anti-phospholipid antibodies that interact with Beta 2 Glycoprotein I (β2GPI) cross-reacts with proteins from several pathogens [74]. Moreover, β2GPI reactive T cells are oligoclonal. Altogether, these data suggest a role for infections in progressive generation and sustaining of the skewed T cell repertoire [75].

A second important factor in progressive distortion of T cell repertoire and autoimmune disease is lymphocyte proliferation following lymphocytic losses, also known as "lymphopenia-driven homeostatic expansion". This is of particular importance since there is a well-known association of autoimmune diseases with lymphopenia, and further, many infections, particularly viral, are also known to lead to lymphopenia [76]. Other factors besides lymphopenia may be involved in generation of pathogenic oligoclonal expansions [77] such as overproduction of IL-21 [76] and depletion of CD4+CD25+ T cells [78]. In addition, local tissue inflammation and persistent antigen burden might work as an important co-factor in autoimmunity generated by lymphopenia [77]. The so-called immune restoration inflammatory syndrome (IRIS) is associated to recent mycobacterial infection prior to highly active antiretroviral therapy (HAART), which restores CD4+ T cell levels and leads to an "autoimmune" syndrome [79]. Noteworthy, HAART is capable of reducing the T oligoclonal pattern in HIV infected patients, along with clinical improvement [69].

Thus, there are similarities between changes of T cell repertoires in infectious and autoimmune diseases: both exhibit oligoclonal expansions of T cells. This has also been registered in allergic diseases: VH gene usage in immunoglobulin E responses of seasonal rhinitis patients allergic to grass pollen is oligoclonal and antigen driven [80].

Bacterial superantigens that are known inducers of massive clonal proliferation and autoimmune-like conditions have also been consistently linked to several autoimmune diseases [81]. Recently, superantigens have been shown to induce *in vivo* and *in vitro* oligoclonal expansions of T cells, reinforcing what we want to propose with another possible mechanism of sustaining T cell repertoire distortion [82].

Vaccination skews oligoclonality

It has been independently shown that vaccination is capable of inducing broad oligoclonal expansions of T cells [83] and autoimmune diseases [84]. Significantly less T cell oligoclonality was found in responders to hepatitis B vaccine, than in non-responders [85]. Other authors have shown that a more polyclonal reactivity is associated with effective hepatitis B vaccination [86].

Particular kinds of worm infections, and also the resident microbiota, have been associated with protection from autoimmune diseases [87] and might explain the epidemiological protection from autoimmune diseases observed in developing countries [88]. As discussed above, some infections are capable of inducing disease-associated oligoclonal expansions of T cells, but other infections are also potent activators of lymphocyte interactions that are responsible for deleting those same oligoclonal T lymphocytes [89].

Intense specific oligoclonal CD4+ T cell expansion follows experimental infection of susceptible Balb/c mice with *Leishmania major*, whereas most other mouse strains, that are resistant to this infection, do not show this expansion. Tolerance-inducing protocols have been shown to increase the resistance of Balb/c mice to Leishmania [90, 91].

Currently, specific immune responses based on clonal expansions of B and/or T lymphocytes are believed to be the fundamental mechanism resulting in protection against infectious diseases. Increased protection achieved by vaccination is believed to derive from the establishment of a progressive, secondary-type responsiveness (memory) allowing more effective immune responses. Although there is abundant *in vivo* and *in vitro* evidence for the association of clonal expansions with immune protection [92], clonal expansions also occur when immune protection is not effective, as repeatedly demonstrated in the many unsuccessful essays of new anti-infectious vaccines, such as against malaria [93] and HIV [94]. Progressive immune responsiveness probably does not explain immunoprotection; rather, it is often associated with pathogeny.

We propose that natural specific immunity and the protective consequences of anti-infectious vaccination derive from assuring clonal diversification and avoiding oligoclonal responses to infectious agents, usually associated with pathogenic infections in susceptible individuals. Infectious, as well as allergic and autoimmune diseases involve perturbations of normally stable state of immune dynamics. Clonal expansions and contractions are probably part of compensatory changes necessary to maintain the steady-state and the invariance of the organization of the immune system. Pathogenic changes in this connectivity may derive from the activation and expansion of a sub-optimal (oligoclonal) diversity of lymphocyte clones. This would explain the association of oligoclonal expansions with a large variety of pathological situations, both in clinical and experimental studies, as well as in examples of inherited immunodeficiencies, such as Omenn syndrome [95, 96].

CONCLUSION

It is widely recognized that under natural conditions only a proportion of the exposed population actually suffers from allergic and infectious diseases. No general explanation has been offered for these differences in individual susceptibility, except for inherited differences that are lacking in many instances. Differences in the degree of oligoclonal lymphocyte expansions could be important in immunopathogenesis and the susceptible individuals would be exactly those exhibiting the most restricted clonality. Our hypothesis also offers a refreshingly new explanation for anti-infectious vaccination: protection would result from an expansion on the clonal diversity triggered by allergic and infectious exposures. Our idea is highly amenable to experimental testing, which is the fundamental value of formulating hypotheses in science.

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